

Quantification of Captopril in Urine Through Surface-Assisted Laser Desorption/Ionization Mass Spectrometry Using 4-Mercaptobenzoic Acid-Capped Gold Nanoparticles as an Internal Standard

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We have developed a new internal standard method for the determination of the concentration of captopril (CAP) through surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) using gold nanoparticles (Au NPs). This approach provided linearity for CAP over the concentration range 2.5–25 μM ($R^2 = 0.987$), with a limit of detection (signal-to-noise ratio = 3) of 1.0 μM . The spot-to-spot variations in the concentration of CAP through SALDI-MS analyses performed in the absence and presence of the internal standard were 26% and 9%, respectively (15 measurements). This approach provides simplicity, accuracy, precision, and great reproducibility to the determination of the levels of CAP in human urine samples. (J Am Soc Mass Spectrom 2010, 21, 864–867) © 2010 American Society for Mass Spectrometry

Captopril (CAP), 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline, is an angiotensin-converting enzyme inhibitor used in the treatment of hypertension and some types of congestive heart failure [1]. Several methods provide good sensitivity and reproducibility for the determination of the concentration of CAP; they, however, require lengthy analyses, high costs, and complicated sampling processes [2, 3].

When performing matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) using nanoparticles (NPs), this approach is often called surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) [4–7]. Several NPs, including Au [8–11], Ag [12], SiO_2 [13, 14], TiO_2 [15, 16], and Fe_3O_4 [17, 18] NPs have been applied in SALDI-MS. Although SALDI-MS provides low variation in the shot-to-shot intensities of MS signals for small solutes (30%; cf. 60% for MALDI-MS), its practical use for the quantitative determination of small solutes in biological samples remains questionable. Expensive and isotope-based labeling reagents are common internal standards for accurate determination of the concentrations of analytes through MS [19, 20].

We developed a simple and rapid internal standard method—SALDI-MS analysis using 4-mercaptobenzoic

acid (MBA)-modified Au NPs (MBA-Au NPs) as an internal standard—for the quantitative determination of CAP in urine. We investigated the roles played by the surface density of MBA on each Au NP, the pH of the solution, and the salt concentration in determining the sensitivity for the analytical procedure. Our proposed method was validated through the quantitative determination of the concentration of CAP in urine.

Experimental

Chemicals

Citric acid, CAP, rhodamine 6G (R6G), salicylazosulfapyridine (SASP), and sodium tetrachloroaurate(III) dehydrate were obtained from Sigma (St. Louis, MO, USA). Mercaptosuccinic acid (MSA), 4-MBA, 11-mercaptoundecanoic acid (11-MUA), 16-mercaptohexadecanoic acid (16-MHA), thioglycolic acid (TA), and trisodium citrate were purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile (ACN) and ammonium hydroxide (NH_4OH) were obtained from Acros (Geel, Belgium). Citric acid (100 mM) and NH_4OH (25%–30%) were used to prepare ammonium citrate solutions (0.05–2 mM, pH 4.00–8.00).

Analysis of CAP in Urine

The 14-nm-diameter Au NPs were prepared [10] and the surface density of thiols on each Au NP was

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determined according to a procedure described previously [21, 22]. Using a calibration curve (fluorescence against the concentration of CAP or MPA), we determined the number of CAP and MPA on each Au NP to be 1750 and 3000, respectively [22]. The urine samples were filtered through 0.45- μm membranes and then spiked with CAP to obtain final concentrations of 50 or 200 μM . Aliquots of the standard solutions of CAP (0–750 μM , 40.0 μL) were added to the urine sample (160 μL). The spiked urine samples (200 μL) were then mixed with 80% ACN (800 μL) and left for 30 min. The proteins in the urine sample were removed through centrifugation at 10,000 rpm for 10 min. The ACN in the suspension solutions (950 μL) was evaporated under vacuum and the resulting pellets were re-dissolved in ammonium citrate (0.1 mM, pH 6.00) to a final volume of 200 μL . The Au NPs (final concentration: 7.5 nM) were added to the protein depleted urine samples (50 μL) and the mixtures incubated at room-temperature for 40 min before SALDI-MS analysis. CAP molecules formed Au–S bonds to the Au NPs; hereafter these solutions are referred to as “CAP–Au NP solutions.”

The detection of CAP in solutions containing CAP (26.25 μM) and 14-nm Au NPs (0.15–75 nM) through SALDI-MS was performed at different concentrations (0.05–2 mM) of ammonium citrate and pH values (pH 4.00, 6.00, 8.00). When applying the internal standard, MBA (225 μM , 50 μL) was added separately to samples of the 14-nm Au NPs (75 nM, 50 μL) in ammonium citrate solution (0.125 mM, pH 6.00, 400 μL). The mixture was equilibrated for 30 min to obtain the MBA–Au NP internal standard solutions (0.5 mL). Equal volumes (50 μL) of the MBA–Au NP internal standard and CAP–Au NP solutions were mixed by vortexing for 1 min. Aliquots (1.0 μL) of mixtures were pipetted onto a stainless-steel 96-well MALDI target and dried in air at room temperature for 1 h before SALDI-MS measurements. Mass spectra were recorded in the negative ion mode using a Microflex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) [10].

Results and Discussion

To improve the accuracy and precision for the determination of CAP through SALDI-MS using Au NPs (7.5 nM), we tested five compounds as candidates for internal standards: 4-MBA, MSA, 16-MHA, 11-MUA, and TA (each concentration: 22.5 μM). All of these potential internal standards bound readily to the surfaces of the Au NPs through Au–S bonding, thereby minimizing their desorption during sample preparation. Because 16-MHA and TA were undetectable at 15 μM , they were unsuitable candidates. Background interference from ionic Au clusters occurred when using MSA and 11-MUA. Under laser irradiation, Au atoms on the Au NP surfaces desorbed and ionized to form ionic Au clusters. MBA provided the lowest background noise and the strongest MS signal; therefore, it became our choice

for use as the internal standard for the determination of CAP.

Figure 1 displays SALDI mass spectra of CAP in the presence of MBA when using Au NPs. The spectrum of MBA and CAP clearly displays peaks at m/z 153.00 and 216.01, respectively. Because the pH of the solution affects the charge densities of CAP and Au NPs, it is an important parameter determining their mutual interactions and the desorption/ionization efficiency of CAP (Figure 2a). Upon increasing the pH value from 4.00 to 6.00, the intensity of the MS signal of CAP increased, mainly because of the increased negative charge density on the Au NP surfaces ($\text{pK}_{\text{a}2}$ and $\text{pK}_{\text{a}3}$ for citric acid are 4.40 and 5.70, respectively) and CAP (pK_{a} 3.70). At values of pH greater than 6.00, the intensity of the MS signal of CAP decreased upon increasing the pH, mainly because of ionization suppression due to the increased ionic strength in the system. The intensity of the MS signal for CAP increased upon increasing the concentration of ammonium citrate from 0.05 to 0.1 mM, due probably to the formation of NH_4^+ /CAP ion pairs and to the improved stability of the CAP-adsorbed Au NPs (i.e., the greater surface area of the monodisperse Au NPs resulted in a greater number of CAP molecules being accessible to ionization during laser irradiation). The ionization efficiency of CAP decreased, however, upon increasing the concentration of ammonium citrate to greater than 0.1 mM, primarily because of ionization suppression effects and the decreased number of CAP molecules adsorbed on the Au NPs. For the 0.1, 0.5, and 1 mM ammonium citrate solutions (pH 6.00), 1750, 1500, and 1000 molecules of CAP, respectively, were bound to each Au NP at saturation. The intensity of the CAP signal was higher in 0.1 mM ammonium citrate solution (pH 6.00).

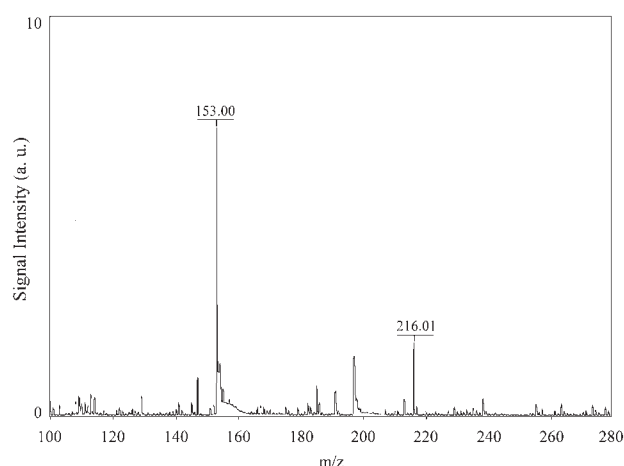


Figure 1. SALDI Mass spectrum of CAP in the presence of MBA. The concentration of each analyte was 22.5 μM . Solution of 14-nm Au NPs (7.5 nM) was prepared in 0.1 mM ammonium citrate (pH 6.00). The peaks at m/z 153.00 and 216.01 correspond to the $[\text{MBA} - \text{H}]^-$ and $[\text{CAP} - \text{H}]^-$ ions, respectively. A total of 150 pulsed laser shots were applied under a laser fluence set at 51.25 μJ .

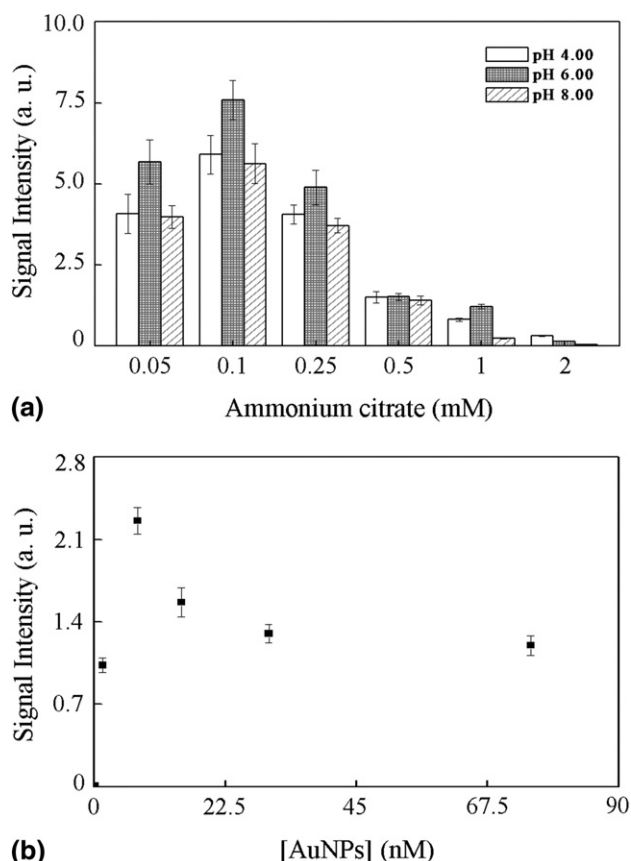


Figure 2. SALDI-MS signals of CAP in solutions (a) at various concentrations of ammonium citrate and pH values, and (b) at various concentrations of Au NPs. In (a), the concentration of Au NPs was 15 nM. In (b), the Au NPs were prepared in 0.1 mM ammonium citrate solution (pH 6.00). Other conditions were the same as those described in Figure 1.

We also investigated the role that the concentration of the Au NPs (0.15–75 nM) played in affecting the determination of the concentration of CAP (26.25 μ M). Figure 2b indicates that the intensity of the signal for CAP was maximized when using a 7.5 nM solution of the Au NPs; at lower concentrations, the intensity was lower because of inefficient ionization/desorption of the analytes. Loss of mass-resolution and stronger background signals became problematic when the concentration of Au NPs was greater than 7.5 nM [10]. Under the optimal conditions (7.5 nM Au NPs), we tested the reproducibility for the determination of CAP through SALDI-MS using MBA-Au NPs as the internal standard. To minimize the adsorption of CAP onto the MBA-Au NPs, we prepared the MBA-Au NPs in 22.5 μ M MBA solution. A fluorescence result (data not shown) shows that the surfaces of the Au NPs (15 nM) were saturated with 45 and 26.25 μ M MBA and CAP, respectively.

Under the optimal conditions (7.5 nM Au NPs; 0.1 mM ammonium citrate; and pH 6.00), we determined the concentrations of CAP in solutions though SALDI-

MS. The intensity of the MS signal of CAP and the MS signal ratio of CAP to MBA were both linear against the CAP concentrations over the range from 2.5 to 25 μ M ($R^2 = 0.987$). The limits of detection (LODs) for CAP in the absence and presence of the MBA-Au NP internal standard at an S/N ratio of 3 were both 1.0 μ M. In addition, the variability of the signal intensities of CAP from 15 different sample spots at different CAP concentrations (5, 10, and 20 μ M) decreased when applying the internal standard; the spot-to-spot variations of the signal intensity of CAP were all less than 26% and 9% in the absence and presence, respectively, of the internal standard.

To demonstrate the practicality of our internal standard SALDI-MS approach, we determined the concentrations of CAP in urine samples. By using a standard addition method and the internal standard, we detected CAP at concentrations of 47.1 ± 1.5 and 192 ± 3.2 μ M in urine samples that had been spiked with 50 and 200 μ M CAP, respectively ($n = 3$); the recoveries were 94.2% and 96%, respectively. The loss of CAP was due mainly to the interactions of CAP with the urine matrix, hydrolysis of CAP, and adsorption of CAP on the vials. The RSD values of three replicate inter-day measurements were 7.1%, 5.7%, and 8.3%, respectively. Relative to separation techniques with fluorescence detection for the determination of the concentration of CAP in urine samples, our approach is simpler (derivatization of CAP with a fluorophore is not required) and requires shorter analysis time (<3 min versus >30 min) [2, 3]. This internal standard SALDI-MS approach improves the accuracy and precision of the determination of CAP. It is thus our strong belief that our approach holds great potential for analysis of small molecules in real samples.

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